REMARKS/ARGUMENTS

This application was originally filed with claims 1-21. Following the first Office Action, claims 15-21 were withdrawn from consideration. In the Final Office Action, claims 1-14 were rejected. In this response after final, claims 15-21 are canceled and claim 2 has been amended solely to correct the dependency and antecedent basis of the claim. No new matter has been introduced by way of the amendment.

Double Patenting Rejection under 35 U.S.C. §101

Claims 1-14 stand provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as that set out by claims 1-5 and 7-11 of co-pending Application No. 10/546,139. This rejection is herein traversed because in response to a restriction requirement issued in the prosecution of co-pending Application No. 10/546,139 on June 20, 2007, claims 1-12 and 15-37 were withdrawn leaving pending only claims 13 and 14. Thus, claims 1-5 and 7-11 of the '139 application can no longer provide grounds for the rejection of claims 1-14 of the instant application. Applicants respectfully request that the rejection be withdrawn.

Claims 1-5 and 7-11 stand provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as that of claims 1-5 and 7-11 of co-pending Application No. 10/546,139. This rejection is now traversed. For the reasons stated above, withdrawal of claims 1-12 of the '139 application no longer provide grounds for the rejection of claims 1-5 and 7-11 of the instant application. Applicants respectfully request withdrawal of the rejection.

NonStatutory Double Patenting Rejection

Claim 6 stands provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1 and 6 of co-pending Application No. 10/546,139. This rejection is now traversed. For the reasons stated above, because of the withdrawal of claims 1-12 of the '139 application, claims 1 and 6 of that application can no longer provide grounds for the rejection of instant claim 6. Applicants respectfully request withdrawal of the rejection.

CLAIM REJECTIONS UNDER 35 U.S.C. §102

Rejections over Nakamori

Claims 1-4 and 8-14 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Nakamori *et al.*, Appl. Micro. Biotech., 52:179-185, 1999 (Nakamori). Applicant respectfully traverses the rejection. Specifically, the cited references "do not teach each and every element as set forth in the claim" MPEP 2131.

Nakamori Does Not Teach The Elements of the Claim

In the Office Action, the Office has incorrectly recited the elements of the claims and identified elements in the cited references that are absent. Specifically, in the instant Action, the Office characterizes the claim as:.

> permitting a modification of metabolic pathways comprising the following steps: a) preparing a modified microorganism by genetic modification of cells of an initial microorganism so as to inhibit the production or consumption of a metabolite when that microorganism is grown on a defined medium, thereby impairing the ability of that microorganism to grow; b) culturing the modified microorganism thereby obtained on said defined medium to cause it to evolve, where the defined medium can contain a co-substrate to allow such evolution; and c) selecting a modified microorganism able to grow on said defined medium, if necessary with a co-substrate.

Final Office action at page 5.

However, in the response to the previous office action mailed on May 16, 2007, claim 1 was amended to read:

> 1. (Currently amended) A method for the preparation of evolved microorganisms ~~permitting a modification of metabolic pathways~~, comprising the following steps: a) preparing a modified microorganism by directed genetic modification of cells of an initial microorganism so as to inhibit the production or consumption of a metabolite when that microorganism is grown on a defined medium, thereby impairing the ability of that microorganism to grow; b) culturing the modified microorganism thereby obtained on said defined medium ~~to cause it to evolve, where~~ wherein the defined medium can

contain a co-substrate ~~to allow such evolution~~; and c) selecting a modified microorganism able to grow on said defined medium, if necessary with a co-substrate, and thereby preparing an evolved microorganism.

Nakamori et al. do not Disclose Directed Genetic Modification

Thus, as recited in claim 1, clause (a) requires, in part, the *directed* genetic mutation of cells of an initial microorganism, thereby impairing the ability of that microorganism to grow. In contrast, Nakamori discloses the induction of random mutagenic events specifically *via* chemical mutagenesis utilizing N-methyl-N'-nitro-N-nitrosoguanidine. Random mutagenesis does not target specific genes or pathways and does not necessarily inhibit the production or consumption of a metabolite nor necessarily impair the ability of the microorganism to grow.

The methods disclosed by Nakamori et al. do not include a ***directed genetic modification*** as is required by instant claim 1. In particular, the Offices' attention is directed to Sugimura et al. (*Nature* 210, 962 - 963 (28 May 1966) and to Shen et al. *J. Proteome Res.*, **5** (2), 385 -395, 2006.

N-Methyl-*N*'-nitro-*N*-nitrosoguanidine (NG) is known to be a potent mutagenic substance for *Escherichia coli* [1], *Salmonella typhimurium* [2], and *Chlamydomonas reinhardi* [3]. Induction of chromosomal aberrations in the cells of root meristems of *Vicia faba* by NG has been reported[4]. It is claimed that NG is the most potent mutagen yet discovered, since NG induced at least one mutation per cell of *Escherichia coli* treated at the optimal condition permitting 50 per cent survival[5]. The production of a number of multi-site mutants resulting from the accumulation of single-site mutations has been noticed. (Sugimura et al., Abstract)

Alkylating agent MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) can induce DNA damages which can lead to chromosomal aberrations, mutations, and cell death. Previous reports from our laboratory have found that low concentration of MNNG can induce nontargeted mutations (NTM) at undamaged bases in DNA, clustering of epidermal growth factor receptor (EGFR) and interference of EGFR mediated signaling, as well as activation of endoplasmic reticulum stress. Thus, the cellular responses to MNNG exposure are very complex, and can be triggered by signals originated from different compartments of the exposed cells. To further probe the molecular mechanisms involved

> in cellular responses to MNNG treatment, and to find potential biomarkers for MNNG induced stress condition, we performed proteomic analysis of whole cellular proteins from human amnion epithelial cells after exposing to MNNG at 3 different doses. More than 80 proteins were affected by MNNG treatment, and 71 proteins among them were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. These proteins take part in a wide variety of cellular processes including regulation of transcription, metabolism, cytoskeleton organization, cell cycle, cell proliferation, signal transduction, transportation, etc. The significance of these proteins in the genesis of MNNG induced cellular defensive response and hazardous effect remains to be elucidated, the results may also give a clue for biomarker search for monitoring the exposure risk of MNNG. (Shen et al., Abstract)

With regard to the actions of N-methyl-N'-nitro-N-nitrosoguanidine, Applicants point out that the use of N-methyl-N'-nitro-N-nitrosoguanidine to promote random mutagenic events is incompatible with claim 1 of the present invention which requires directed genetic modification. For this reason, at least, the rejection should be withdrawn.

Nakamori et al. do not Obtain a Modified Microorganism which is then Cultured

Further, the instant specification teaches that a 'modified microorganism' is "a microorganism obtained by performing controlled modifications, i.e., that are not the result of a process of evolution. Examples of such a modification are the direct mutation or deletion of a gene, or the directed modification of a promoter." Paragraph [0038]. A variety of methods for the directed modification of a microorganism are described in the specification. For example, some described methods include gene attenuation, deletion of the desired gene or genes in the metabolic pathway that is desired to be evolved. Paragraph [0050]. Specific examples of methods to overexpress a gene includes the overexpression of heterologous or nonheterologous genes. Paragraph [0051]. Methods to achieve such overexpression by, for example, replacing the promoter of the gene *in situ* by a strong or inducible promoter or by a single-copy or multi-copy replicative plasmid in which the gene is overexpressed (paragraph [0052] and such modification can be decided on a case by case basis (paragraph [0053]). The specification also describes methods to inactivate a gene, such as by, for example, homologous recombination. Paragraph [0055].

As recited in the claim, the modified microorganism must be 'obtained' before it can be cultured. Using the techniques above, as discussed in the specification, the modified microorganism is obtained by being selected, e.g., being resistant to and being grown in the presence of a deleterious compound such as an antibiotic. Such resistance markers are known and include ampicillan resistance, kanamycin resistance, chloramphenicol resistance and the like. The ability of the microorganism to grow in the presence of an antibiotic, for example, allows the modified microorganism to be obtained. Once the modified microorganism is obtained it can be cultured on a defined medium, e.g. a medium " substantially free of the metabolite or metabolites, the production of which is inhibited by performing the modification." Paragraph [0021].

In contrast, Nakamori et al. describe the random mutagenesis of *E. coli* via *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine to obtain analog resistant mutants that were L-methionine producers, "[T]able 1 shows the results of deriving L-methionine producers from L-methionine –analogue-resistant mutants." Nakamori et al., pg. 181, second column. Therefore, Nakamori et al. do not "obtain" "modified" bacteria and the mutant bacteria that Nakamori et al. produce are immediately identified by their ability to produce L-methionine. The mutant bacteria produced by Nakomori et al. are not further cultured on defined media. Thus, Nakamori et al. lack the elements of steps (b) and (c) of claim 1. Therefore, for this reason, at least, the rejection is in error and should be withdrawn.

Thus, for at least the reasons stated above, Nakamori et al. fail to teach the elements of claim 1 and therefore cannot anticipate claim 1 and those claims depending therefrom. Thus, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

Rejections over WO 93/17112 to Lievense

Claims 1-7 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by WO 93/177112 to Lievense. Applicant respectfully traverses the rejection because Lievense does not teach or disclose all the elements of the claim. Specifically, as recited above, claim 1, *requires* at least the elements (a) preparation of a modified microorganism by directed genetic modification, impairing the ability of the microorganism to grow on a defined medium; (b) culturing the

modified microorganism; and (c) selecting a modified microorganism whereby an evolved microorganism is prepared.

Lievense Does Not Teach Preparing a Modified Microorganism that is Impaired in Its Ability To Grow

The present invention teaches a directed genetic modification which results in a modified organism having a genetic modification "thereby impairing the ability of that microorganism to grow". The introduced deficiency is complimented organically in order to rescue the modified microorganism. In contrast, Lievense merely inserts foreign genes in bacteria allowing the bacteria to used non-native substrates to produce methionine. The growth of the transformed bacteria taught by Lievense is not impaired.

In particular, the Offices' attention is directed to Tables I-VII of Lievense which show that the controls grow on the substrate but merely do not produce methionine (Tables I, III-VII) or homocystein (Table II) as the transformants do. For this reason the rejection is overcome.

Further, claim 1 also requires that the modified microorganism is cultured on a defined medium. However, applicants direct the Offices' attention to Example 1 of Lievense describing "the parent and transformed microbes are cultivated individually in a fermentation medium containing glucose, soy hydrolysate, and inorganic nutrients. The medium is supplemented either with sulfate or sulfide as a source of sulfur for methioonine production." WO93/17112 at 7. Applicants point to one description of soy hydrolysate from Millipore, Inc.:

> **Description:** LucraTone Soy F hydrolysate is obtained from the enzymatic hydrolysis (non-animal) of defatted soybean plant flour from Identity-Preserved (IP) sources as it pertains to GMOs. It is fortified with essential amino acids and vitamins.
> **Certification:** Animal-free, GMO free
> **Applications:** LucraTone Soy F supplement is a highly productive hydrolysate with a high carbohydrate content and large quantities of smaller molecular weight peptides. It is easily assimilated by many microorganisms and is a good source of nitrogen for cell culture medium or fermentation substrates.

(http://www.millipore.com/publications.nsf/docs/tb1036en00)

Applicants therefore submit that any medium containing soy hydrolysate is not a defined medium as required by claim 1. Thus, for this reason, Lievense cannot anticipate the present invention and the rejection is overcome.

In addition, the Offices' attention is drawn to paragraph [0074]:

> The application of the invention to the modification of the biosynthesis pathway of methionine requires first genetically modifiying the initial bacterium (step (a)). This modification necessarily involves the attenuation of at least one gene and possibly the cloning of at least one heterologous gene. The attenuation of the gene must make the bacterium depend on the restoration of an equivalent metabolic pathway to allow it to grow (step (b)). The genetically modified bacterium is grown, and the bacterial strain or strains whose growth improves in the presence or absence of an exogenous co-substrate are selected (step (c)).

These general strategies are followed by specific examples in section 'F' e.g., paragraphs [0197]-[0481]. Specifically, the Offices' attention is directed to, for example, paragraph [0198] describing the deletion of the metE gene (including selection of the modified strain on defined media) followed by [0199] culture of the selected modified strain on minimal media and [0200] isolation of the evolved gene [0201]. Example F.I.1 gives one specific example of the construction of the modified strain of *E. coli* wherein the metE gene is deleted by insertion of a disrupted heterologous gene bearing a chloramphenicol resistance cassette. The modified organism is then selected for by its ability to grow in chloramphenicol containing media. Paragraphs [0210]-[0221]. Once the modified strain is selected, the *ΔmetE* strain is then grown in media containing methylmercaptide, to select a strain that can make methionine using methylmercaptan as a source. Paragraph [0225]. The evolved organism is then selected by its ability to use sodium methylmercaptide as a source. Applicants do not teach transformation with a gene in trans that allows it to utilize methylmercaptan as does Lievense.

Lievense only describes the synthesis of methionine by the transformation or transfection of *E. coli* with plasmids bearing various genes in the methionine synthesis pathway. Lievense, thus, only teaches part of step (a) of the present invention, e.g. the preparation of a modified microorganism. Lievense does not disclose a microorganism that is impaired in its ability to grow, as illustrated by Tables I-VII. Lievense does not teach the evolution of native pathways to rescue the modified microorganism growth. Lievense does not teach either step (b) or step (c) of claim 1.

Thus, at least for these reasons, Lievense cannot anticipate claim 1 and all claims depending therefrom. Therefore, applicants respectfully request that the rejection be withdrawn and the claims passed to allowance.

CONCLUSION

In view of the above, Applicant respectfully submits that the present application is in condition for allowance. Reconsideration of the present application and a favorable response are respectfully requested.

Respectfully submitted,

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